(FILE 'HOME' ENTERED AT 07:41:49 ON 30 APR 2004)

FILE 'MEDLINE, CANCERLIT, EMBASE, BIOSIS, BIOTECHDS, CAPLUS' ENTERED AT 07:42:10 ON 30 APR 2004

L1	72048	S	FACTOR VIIA OR FACTOR VII OR FACTOR VIII OR FACTOR IX
L2	2382129	S	PLASMID OR POLYMER OR MICROPARTICLE OR LIPID OR LIPOSOME OR C
L3	3170	S	L1 AND L2
L4	148610	S	GENE THERAPY
L5	522	S	L4 AND L3
L6	428		DUP REM L5 (94 DUPLICATES REMOVED)
L7	4076186	S	DOSE OR DOSAGE OR AMOUNT
L8	157	S	L7 AND L6
L9	3453425	S	REVIEW
L10	4	S	L9 AND L6
L11	545823	S	HEMOPHILIA OR COAGULATION OR BLEEDING
L12	220	S	L11 AND L6
L13	56	S	L12 AND L7
L14	52	S	L13 AND PLASMID

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L10 ANSWER 2 OF 4 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
AN 2001-03389 BIOTECHDS
TI Gene transfer as an approach to treating hemophilia; retro virus, **plasmid**, adeno-associated virus, adeno virus or lenti virus vector-mediated **Factor-IX** gene transfer and expression in skeletal muscle or liver for disease **gene therapy**; a **review**
AU High K A
CS Univ.Pennsylvania; Child.Hosp.Philadelphia
LO The Children's Hospital of Philadelphia, 3516 Civic Center Blvd, 310 Abramson Research Center, Philadelphia, PA 19104, USA.
Email: high@email.chop.edu
SO Circ.Res.; (2001) 88, 2, 137-44
CODEN: CIRUAL ISSN: 0009-7330
DT Journal
LA English
AB The use of gene transfer as an approach to treating hemophilia is reviewed. Present treatment for hemophilia involves i.v. infusion of either recombinant or plasma-derived clotting factor concentrates. However, this method of treatment is expensive and there are risks of blood-borne disease transmission. Hemophilia has a number of advantages as a model system for working out strategies for gene transfer as an approach to the **gene therapy** of genetic diseases, which include: wide lattitude in choice of target tissue; broad therapeutic window for levels of circulating factor; ease of determining therapeutic endpoints; and existence of excellent animal models of the disease. Three clinical trials, each using different vectors and target tissues, are currently in progress, and 2 additional trials are in late planning stages. Strategies of **gene therapy** and gene transfer for hemophilia therapy have included using: retro virus-mediated approaches; **plasmid**-based approaches; adeno-associated virus vector approaches expressing **Factor-IX** to skeletal muscle or the liver; adeno virus-mediated approaches; and lenti virus-mediated approaches. (70 ref)

L14 ANSWER 42 OF 52 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
AN 2003-07322 BIOTECHDS
TI New non-viral vesicle vector comprises vesicular membrane with hepatitis B envelope protein and nucleic acid expression construct comprising complete **factor VIII** or IX coding sequence, useful for treating **hemophilia**;
vector-mediated gene transfer and expression in host cell useful for **hemophilia gene therapy**
AU CHIEN K R; HOSHIJIMA M
PA UNIV CALIFORNIA
PI WO 2002086091 31 Oct 2002
AI WO 2002-US13164 25 Apr 2002
PRAI US 2001-286314 25 Apr 2001; US 2001-286314 25 Apr 2001
DT Patent
LA English
OS WPI: 2003-093125 [08]
AB DERWENT ABSTRACT:
NOVELTY - Non-viral vesicle vector comprising: (a) a vesicular membrane with hepatitis B envelope protein exposed on the vesicle surface; or (b) a nucleic acid expression construct comprising a complete **factor VIII** or **factor IX** coding sequence and a promoter sequence functional in liver cells, is new.

DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for treating **hemophilia**.

BIOTECHNOLOGY - Preferred Vector: The envelope protein of the non-viral vesicle vector contains mutations to reduce antigenicity. The expression construct is double stranded **plasmid** DNA or RNA and comprises inverted terminal repeat sequences from adeno-associated virus (AAV-ITR), eukaryotic transposon, transposase sequences, the coding sequence of **factor VIII** or IX. The **factor VIII** comprises silent mutations to enhance expression. The promoter is a non-tissue specific promoter comprising cytomegalovirus, Rous sarcoma virus, ubiquitin, chicken beta-actin or elongation factor 1alpha promoter, or preferably liver specific promoter. The liver specific promoter comprises alpha-fetoprotein promoter, globulin promoter, approximately1-microglobulin or albumin. Preferred Method: Treating **hemophilia** comprises: (a) administering into circulation of an individual with **hemophilia** the non-viral vesicle vector and the nucleic acid expression construct; and (b) monitoring the individual for amelioration of disease. Administration into circulation comprises intravenous or intraarterial administration, particularly into hepatic or portal artery.

ACTIVITY - Hemostatic. No suitable data given.

MECHANISM OF ACTION - **Gene therapy**.

USE - The non-viral vesicle vector is useful for treating **hemophilia** (claimed).

ADMINISTRATION - The non-viral vesicle vector and the nucleic acid expression construct is administered via intravenous or intraarterial route, particularly into hepatic or portal artery (claimed). No **dosage** given. (34 pages)

L14 ANSWER 4 OF 52 MEDLINE on STN
AN 2001231742 MEDLINE
DN PubMed ID: 11319920
TI **Gene therapy** for the treatment of **hemophilia** B using PINC-formulated **plasmid** delivered to muscle with electroporation.
AU Fewell J G; MacLaughlin F; Mehta V; Gondo M; Nicol F; Wilson E; Smith L C
CS Valentis, Inc., The Woodlands, Texas 77381, USA.
SO Molecular therapy : journal of the American Society of Gene Therapy, (2001 Apr) 3 (4) 574-83.
Journal code: 100890581. ISSN: 1525-0016.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200107
ED Entered STN: 20010730
Last Updated on STN: 20010730
Entered Medline: 20010726
AB **Gene therapy**, as a safe and efficacious treatment or prevention of diseases, is one of the next fundamental medical innovations. Direct injection of **plasmid** into skeletal muscle is still a relatively inefficient and highly variable method of gene transfer. However, published reports have shown that application of an electric field to the muscle immediately after **plasmid** injection increases gene expression at least 2 orders of magnitude. Using this methodology, we have achieved potentially therapeutic circulating levels of human **factor IX** (hF.IX) in mice and dogs. A **plasmid** encoding hF.IX formulated with a protective, interactive, noncondensing (PINC) **polymer** was injected into the skeletal muscle followed by administration of multiple electrical pulses (electroporation). In mice long-term expression was achieved and the ability to readminister formulated **plasmid** was demonstrated. In normal dogs, expression of hF.IX reached 0.5-1.0% of normal levels. The transient response in dogs was due to the development of antibodies against hF.IX. Elevated circulating creatine kinase levels and histological examination indicated transient minor trauma associated with the procedure. These data show that gene delivery using a **plasmid** formulated with a PINC **polymer** augmented with electroporation is scalable into large animal models and represents a promising approach for treating patients with **hemophilia** B.

L14 ANSWER 3 OF 52 MEDLINE on STN
AN 2001253005 MEDLINE
DN PubMed ID: 11273783
TI Linear DNAs concatemerize in vivo and result in sustained transgene expression in mouse liver.
AU Chen Z Y; Yant S R; He C Y; Meuse L; Shen S; Kay M A
CS Department of Pediatrics, Stanford University School of Medicine, Stanford, California 94305, USA.
NC DK49022 (NIDDK)
SO Molecular therapy : journal of the American Society of Gene Therapy, (2001 Mar) 3 (3) 403-10.
Journal code: 100890581. ISSN: 1525-0016.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200108
ED Entered STN: 20010806
Last Updated on STN: 20010806
Entered Medline: 20010802
AB The short duration of transgene expression remains a major obstacle for the implementation of nonviral DNA vectors in clinical **gene therapy** trials. Here, we demonstrate stable, long-term transgene expression in vivo by transfecting a linear DNA expression cassette (LDNA) into mouse liver. Interestingly, despite similar quantities and cellular distribution of injected DNAs in their livers, mice receiving LDNA encoding human alpha1-antitrypsin (hAAT) expressed approximately 10- to 100-fold more serum hAAT than mice injected with closed circular (cc) DNA for a period of 9 months (length of study). Furthermore, when a linear human **factor IX** expression cassette was delivered to **factor IX**-deficient mice, sustained serum concentrations of more than 4 microg/ml (80% of normal) of the human clotting factor and correction of the **bleeding** diathesis were obtained. Southern blot analyses indicate that, unlike ccDNA, LDNA rapidly formed large, unintegrated concatemers in vivo, suggesting that transgene persistence from **plasmid**-based vectors was influenced by the structure of the vector in transfected cells. No differences in transgene expression or DNA molecular structures were observed when AAV ITRs were included to flank the hAAT expression cassette in both ccDNA- and LDNA-treated animals. Linear DNA transfection provides an approach for achieving long-term expression of a transgene in vivo.

L14 ANSWER 2 OF 52 MEDLINE on STN
AN 2001345700 MEDLINE
DN PubMed ID: 11407909
TI Long-term and therapeutic-level hepatic gene expression of human **factor IX** after naked **plasmid** transfer in vivo.
AU Miao C H; Thompson A R; Loeb K; Ye X
CS Puget Sound Blood Center, University of Washington, Seattle, Washington 98104, USA.. miao@u.washington.edu
SO Molecular therapy : journal of the American Society of Gene Therapy, (2001 Jun) 3 (6) 947-57.
Journal code: 100890581. ISSN: 1525-0016.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200108
ED Entered STN: 20010903
Last Updated on STN: 20010903
Entered Medline: 20010830
AB Naked DNA transfer of a high-expressing human **factor IX** (hFIX) **plasmid** yielded long-term (over 1 1/2 years) and therapeutic-level (0.5-2 microg/ml) gene expression of hFIX from mouse livers. The expression cassette contained a hepatic locus control region from the ApoE gene locus, an alpha1-anti-trypsin promoter, hFIX cDNA, a portion of the hFIX first intron, and a bovine growth hormone polyadenylation signal. In contrast, a hFIX **plasmid** containing the expression cassette without effective regulatory elements produced initially low-level gene expression that rapidly declined to undetectable levels. Southern analyses of the cellular DNA indicated that the majority of the input genome from either vector persisted as episomal forms of the original plasmids. Together with RT-PCR analyses of the transcripts, these data indicated that at least two processes are critical for sustained gene expression: persistence of vector DNA and transcriptional/posttranscriptional activation. Liver regeneration after partial hepatectomy resulted in a significant decline in transgene expression, further suggestive of decreased episomal **plasmid** maintenance rather than transgene integration. Transaminase levels and liver histology showed that rapid intravenous **plasmid** injection into mice induced transient focal acute liver damage (< 5% of hepatocytes), which was rapidly repaired within 3 to 10 days and resulted thereafter in histologically normal tissue. No significant differences were observed between rapid injection of **plasmid** and saline control solutions. Transient, very low level antibodies directed against hFIX did not prevent the circulation of therapeutic levels of the protein. Gene transfer of hFIX **plasmid** DNA into liver elicited neither transgene-specific cytotoxic effect nor long-term toxicity. These results demonstrate that long-term expression of hFIX can be achieved by nonviral **plasmid** transfer and suggest that this occurs independent of integration.

L14 ANSWER 6 OF 52 MEDLINE on STN
AN 95394346 MEDLINE
DN PubMed ID: 7665069
TI **Liposome**-encapsulated DNA-mediated gene transfer and synthesis of human **factor IX** in mice.
AU Baru M; Axelrod J H; Nur I
CS Octa Medical Research Institute, Kiryat Weizmann, Rehovot, Israel.
SO Gene, (1995 Aug 19) 161 (2) 143-50.
Journal code: 7706761. ISSN: 0378-1119.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199510
ED Entered STN: 19951020
Last Updated on STN: 19990129
Entered Medline: 19951006
AB **Hemophilia** B is an X-chromosome-linked recessive disorder that is caused by a deficiency of biologically active clotting **factor IX** (FIX). In this work, liposomes (Lip) were used for non-viral, in vivo gene transfer of the human FIX gene into mouse organs. **Plasmid** DNA, containing the human FIX cDNA under the control of the Moloney murine leukemia virus (MoMLV) long terminal repeat (LTR), was encapsulated in 1-2-microns multilamellar Lip composed of egg phosphatidylcholine (EPC). The percentage of Lip-associated DNA was 47%, and 72% of the Lip DNA was protected from DNase I digestion. The Lip-encapsulated (Len) DNA was injected intravenously into Balb/c mice, and at various times post-injection, various tissues were examined for the presence of the exogenous DNA. **Plasmid** DNA was detected by Southern blot analysis mainly in the liver and spleen, but small amounts were also detected in the lungs, heart and kidneys. The **plasmid** DNA was retained in mouse liver cells for at least 7 days post-injection, and remained in an episomal state. The levels of human FIX protein in the mouse plasma were 190-650 pg per ml for 2 to 7 days post-injection. Treatment of mice with chloroquine (Cq) and colchicine (Cc) prior to Lip injection significantly increased the **amount** of **plasmid** DNA found in the liver cells, as well as the level of human FIX in the plasma. These results demonstrate the potential use of Len DNA for gene transfer into liver and spleen, and for **gene therapy** of inherited and acquired disorders.